K68 Blood Clonazepam and 7-Aminoclonazepam Trends in Postmortem and Driving Under the Influence of Drugs (DUID) Cases

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After attending this presentation, attendees will understand the trends of both parent clonazepam and the primary metabolite/degradant 7-aminoclonazepam in blood from postmortem and DUID cases.

This presentation will impact the forensic science community by illustrating the need to include 7-aminoclonazepam as a marker in the analysis of blood samples to help determine the likelihood of clonazepam exposure.

Clonazepam is a commonly encountered benzodiazepine in postmortem and DUID toxicology. It is routinely prescribed for the treatment of seizure and panic disorders and may also be abused for its sedative, hypnotic, and anxiolytic properties. Often, laboratory analysis of blood samples will only measure the parent analyte clonazepam. Clonazepam is extensively metabolized to 7-aminoclonazepam, an active metabolite, by the reduction of the 7-nitro group. The instability of the 7-nitro group of parent clonazepam may also result in post-sample collection or postmortem formation of the 7-amino analog. For these reasons, this study seeks to demonstrate that 7-aminoclonazepam should be a requisite analyte included in the analysis of blood samples as a marker for clonazepam use.

A confirmatory Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method for several common benzodiazepines, which included clonazepam and 7-aminoclonazepam, was developed and validated. Samples were prepared by protein precipitation followed by a supported liquid extraction. High-Pressure Liquid Chromatographic (HPLC) separation was performed on a biphenyl column. Positive ion detection was performed by a triple quadrupole MS/MS operating in Multireaction Monitoring (MRM) mode.

More than 6,000 blood samples collected from postmortem and DUID cases across an eight-month time period were analyzed for the presence of benzodiazepines. The samples were first screened by a validated immunoassay process and non-negatives confirmed by the validated LC/MS/MS method at a threshold of 5ng/mL. Confirmed positives for clonazepam and/or 7-aminoclonazepam were evaluated based on concentration, case type (postmortem vs. DUID), drug co-positivity, etc.

Five hundred ninety-seven samples (9.7%) confirmed positive for one or both clonazepam markers. While the majority of the positive samples confirmed for both parent and metabolite/degradant (74.9%), 144 samples (24.1%) were detected above the threshold for 7-aminoclonazepam only. Parent clonazepam with no 7-aminoclonazepam present was detected in only six samples (1.0%). No distinction was obvious between case type for the detection of 7-aminoclonazepam only. The cause of metabolite/degradant-only results could be due to the possibility of differences in time since ingestion, metabolic variations, potential drug-drug interactions, or degradation due to instability; however, regardless of source, the addition of 7-aminoclonazepam as a marker for clonazepam use appears to provide supporting information that could be valuable to the interpretation of the toxicology results.

7-Aminoclonazepam, Postmortem, DUID

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